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DEPARTMENT OF BIOLOGY

Salicylic Acid Induced Alteration in Growth and Physiological Attributes of Cowpea [*Vigna unguiculata* (L.) Walp.] Cultivars Grown Under Salinity Stress

A Thesis Submitted to Department of Biology, College of Natural and Computational Science, University of Gondar in Partial - Fulfillment of the Requirements for the Master of Science in Botanical Sciences

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List of Acronyms/Abbreviation

DW Dry weight

FC Field capacity

FM Maximum fluorescence

FO ground value of chlorophyll fluorescence

FV Variable florescence

FW Fresh weight

FV/FM Maximum quantum yield of PSII

FYM Farmyard manure

ROS Reactive oxygen species

RWC Relative water content

SA Salicylic acid

TW Turgid weight

Abstract

Salinity is one of the most severe environmental factors limiting the productivity of agricultural crops;SA induces a protective effect on plants under certain adverse environmental conditions.In the present study, the ameliorative role of salicylic acid (SA) against salt stress in the three culivars of cow pea(Vigna *anguiculata,* ILRI 9334,ILRI 9333 andILRI 1114), various parameters of plant growth, water status and chlorophyll fluorescence (Fv/Fm) were analyzed in control and salt-treated (50, 100 and 150 mM NaCl) plants with and without foliar application of 1mM SA. Results revealed significant differences among the cultivars, salt-stress treatments, and their interaction, indicating the cultivars' variability and differential response to salt stress. Salinity stress adversely affected the plant growth, plant water status and Fv/Fm.These relatively less declinesin growth, water status,relative water content and Fv/Fm of cultivar ILRI9334exhibits a reasonable tolerant cultivar, while the other two varieties viz., ILRI1114and ILRI9333proved to be sensitive to salt stress. Moreover, the combined treatments of salt stress and SA promoted growth, plant water status and Fv/Fm.In short, salinity hampered the overall performance of *cow pea* cultivar, but SA application fortified the salt-tolerance capacity mainly in the cultivarILRI 9334.SA applicable, may be by activating its defense arsenal, alleviating the membrane injury, accelerating assimilatory activities and improving plant water status.

Key words/phrase: Biomass,Cowpea accesion, Growth, Maximum quantum yield of PSII, Tolerance,

1. Introduction

Soil-salinity stress is one of the most serious abiotic threats to distribution, survival and productivity of plants. It can disturb a number of biochemical and physiological processes and limit biomass accumulation, which determines the net primary production and growth rate (Tackenberg, 2007; Arshi*et al.,* 2006; 2012; Qureshi *et al.,*2013; Qiong *et al.,* 2016; Ali and Rab 2017).Salinity reduces water-absorption ability of plants, and induces metabolic changes similar to those caused by water stress (Hasegawa *et al.,*2000). The adverse effects on plant growth may be due to ion cytotoxicity and osmotic stress, which cause nutritional deficiencies and metabolic imbalance (Zhu *et al.,* 2002; Arshi *et al.,* 2010a, b; Qureshi *et al.,* 2013).

Salinity affects about 34 million hectares of land (11% of irrigated area) in the world; an additional 60-80 million hectares are affected by water logging and consequent salinity (Anon, 2012). The salt-affected soils are common in the Rift Valley, Awash Valley and lowland areas of Ethiopia (Gebreselssie, 1993). Nearly 57% of the 4000 ha irrigated land of Melka Sadi Farm (Taddese and Bekele, 1996), entire Melka Werer Research Farm (Haider *et al.,* 1988), and 30% of the Abaya State Farm (Tsige *et al.*,2000) are salt-affected. Salinity problem is likely to be more severe in the coming years due to the absence of suitable management practices and growing tendency of large-scale-irrigation agriculture (Mamo *et al.,* 1996).

Salicylic acid (SA) is naturally occurring plant hormone produced commonly asphenolic compound and can act as growth regulator. This compound influences in a variable manner; inhibiting certain processes and enhances the other one. SA influences a range of diverse processes in plants, including seed germination,ion uptake and transport. More interests have been focused on SA due to its ability to induce a protective effect on plants under certain adverse environmental conditions. It is well known fact that SA controls tolerance to salinity as it creats osmotic stress, mineral deficiency and oxidative stress(Saeidnejad *et al*., 2012; Hadi *et al*., 2014; Chaparzadeh and Hosseinzad-Behboud, 2015). However,effect of SA depends on its concentration, plant species, developmental stage, mode of application (Tari *et al*., 2015) and environmental conditions (Salehi *et al*., 2011).

Cowpea [*Vigna unguiculata* (L.)Walp.]is one of the multifunctional crops, providing food for man and livestock and serving as valuable and dependable revenue generating commodity for farmers and grain traders. It has high value of protein content and also more drought tolerant than other leguminous plants. It belongs to family fabaceae and all the cultivated cowpeas are grouped under the species *V. unguiculata* (Ashebir *et al.*, 2013). Among all legumes, cowpea has the maximum diversityfor plant type, growth habit, maturity, seed type and adapted toa wide range of environments which may serve as a model legume crop (Singh, 2005; Hall, 2004). In addition, cowpea is a traditional source of livelihood to many rural African populations. Thus, the wide range of variation exists among different crop plants and their cultivars may be utilized gainfully for identifying and developing the salt-tolerant candidates. Given this, three cowpea cultivars were selected for study of their response to salt stress, assuming that they could show differential capacity of tolerance as affected by SA.

2. Literature Review

2.1. Effect of salt steress on plants

In the recent past, many cultivated landareasare affected by salinity stress and this phenomenon is increasing day to day as the population grows. Thus, one of the greatest challenges in the coming years is to maintain the plant productivity in the saline affected areas, it is vital to understand the mechanism of salt toxicity in plants and find out some tolerant plants or cultivars. Plants respond to salt stress by exhibiting reduction in growth which is usually interlinked with an array of physiological, biochemical and molecular characteristics (Bartels and Sunkar 2005; Munns and Tester, 2008; Park*et al*.,2016; Husen *et al*.,2016, 2017; Albaladejo*et al*.,2017; Negrão*et al*.,2017).

Salinity messed up water uptake and leads to nutrient imbalance due to accumulation of $Na^+$and Cl^- occurring concomitantly with a decrease of K^+(Munns and Tester 2008). In general, high salinity has an adverse effect on various plant growth features and causes stomatal closure, decline of pigment content leading to reduced photosynthesis also increased generation of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen, which damage the cellular components (Qureshi *et al.,*2013; Srivastava *et al*.,2015; Zhang *et al*.,2016; Choudhury *et al*.,2016).

Photosynthetic rate varies with low to high concentration of salinity exposure which is considered as one of the most significant factors restricting plant growth rate (Husen *et al*.,2016, Hussein*et al*.,2017). Plant growth and productivity under salinity stress are also reflected by water use efficiency (Huez-López *et al*.,2011; Husen *et al*.,2017). Chen and Murata (2011) have reported reduced photosynthesis under salt-stress condition by inhibiting photosystem II complex at both acceptor and donor side and thus destruction of chlorophyll pigments by accumulation of toxic ions. These alterations can be measured by a non-destructive technique, namely chlorophyll fluorescence (*Fv/Fm*). It has been reported that measuring the integrity of photosynthetic apparatus driving the photosynthetic process within leaf by *Fv/Fm* provides a rapid and accurate technique of detecting and quantifying plant tolerance to abiotic stresses (Baker 2008; Getnet *et al*.,2015; Husen *et al*.,2014, 2017).

2.2. Stress impacts on membrane permeability

Salinity stress caused an increase in membrane permeability of theplants. Plasma membranes are the first receptors of stress and show both increase and decrease in membrane fludiry increase in plasmalemma unsaturation under salt stress plants indicate an increase in the fluidity of the membrane surface, which could facilitate the deep penetration of reactive oxygen species into the cell membranes, they can protect the cells through modifications affecting stress perception and rigidity of the cell structure(Alpaslan and Gunes, 2001; Filek *et al*., 2012).

Plasma membrane lipid peroxidation highly affected by salt steress due to its effect on permeability of plants which in turn modulates the pattern of ion leakage.Decrease in membrane stability reflects the extent of lipid peroxidation caused by ROS(Ashraf and Ali, 2008)

2.3. Role of Salicylic acid in plants

It is known that exogenous application of plant growth regulators plays a vital role in signaling network, developmental and adaptation processes of various plant species against biotic and abiotic stresses (Cao *et al*.,2007; Bajguz and Hayat 2009; Qiun*et al*.,2014; Fahad *et al*.,2014; Khan *et al*.,2015; Wani *et al*.,2016; Husen *et al*.,2016, 2017). Salicylic acid (SA) is a phenolic growth regulators and involved in plant defence mechanisms against stress like salinity (Fahad *et al*.,2014; Jayakannan *et al*.,2015; Gharbi *et al*.,2016), drought (Nazar*et al*.,2015), ultraviolet light (Bandurska and Cieślak 2013; Li*et al*.,2014), heat (Shi*et al*.,2006), high temperature (Hayat *et al*.,2009), heat and high light stress (Wang*et al*.,2014) plant pathogenesis (Wang *et al*.,2007), and heavy metals toxicity (Song*et al*.,2014; Hayat *etal*., 2014; Namdjoyan *et al*.,2017). Hao *et al*., (2012) have reported that SA-induced expression of 59 proteins in *Cucumis sativus* which were identified for their involvement in a variety of cellular responses and metabolic processes, as well as antioxidative reactions, cell defense, photosynthesis, carbohydrate metabolism, respiration and energy homeostasis, protein folding and biosynthesis. SA under salinity was found to stimulate salt tolerance in maize via accelerating their photosynthetic rate and carbohydrate metabolism (Khodary 2004). Under salinity, mungbean plants have shown reduced photosynthetic process which was adjusted by SA due to induced nitrate reductase activity and ATP-sulfurylase and antioxidant metabolism (Nazar *et al*.,2011). In *Arabidopsis*, SA pre-treatmentssuppressed the adverse effect of

salinity by decreasing K^+ leakage in root tissues and by enhancing the H^+-ATPase activity (Jayakannan *et al*.,2013), that facilitates a driving force for Na^+/H^+ exchanger at the plasmamembrane and directs to reduced sodium accumulation in cytosol (Shi *et al*.,2000). In barley, SA is believed to facilitate biosynthesis of soluble proteins, thus improving plant adaptation under cold stress (Mutlu *et al*.,2016). SA treatment also reduced lipid peroxidation and membrane permeability under salinity (Horváth *et al*.,2007). SA treatments may interact with other plant hormones and play an important role in resistance and or tolerance enhancement of various plant species. Some studies have explored the role of SA in developing plant tolerance to salt stress for instance, *Arabidopsis thaliana*(Jayakannan *et al.,*2013; Horváth *et al.,*2015),*Brassica juncea* (Yusuf *et al*.**,**2008; Syeed*et al*.**,**2011), *Hordeum vulgare* (Fayez and Bazaid 2014; Pirasteh-Anosheh *et al*.**,**2014), *Medicago sativa* (Palma *et al*.,2013), *Oryza sativa*(Jini and Joseph 2017*),Solanum lycopersicum* (Szepesi*et al*. 2009; Gharbi *et al*.,2016; Mimouni *et al*.,2016),*Solanum chilense* (Gharbi *et al*.,2016),*Triticum aestivum* (Li *et al*.,2013),*Vicia faba* (Azooz, 2009) and *Vigna radiata* (Khan *et al*.,2014).

2.4. Antioxidative defense system

Salinity leads to oxidative stress due to imbalance between antioxidant defenses and reactive oxygen species (ROS) levels. Through mitochondria and chloroplasts electron transport systems can generate ROS such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet oxygen ($_1O_2$) (Noreen *et al*., 2009).ROS leads to chlorophyll degradation and membrane lipid peroxidation (measured as malondialdehyde content), reducing membrane fluidity and selectivity (Koyro *et al*.,2013). All these alterations are considered as symptoms of oxidative damage. Thus, to alleviate the harmful effects of these ROS level under stress condition, plants have developed a series of enzymatic and non-enzymatic antioxidative defense system to protect cells from oxidative damage (Husen, 2010; Yousuf *et al*.**,**2015a).

Antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) function, by catalyzing the decomposition of oxidants and free radicals. It has been reported that salinity provoked in plant leaves an imbalance between ROS production and antioxidant defenses, with the induction of oxidative stress (Saikachout *et al*., 2013).The antioxidant enzyme system constitutes SOD as the primary step of cellular defense. It dismutates superoxide ions (O_2^-) to H_2O_2 and O_2. Further, the accumulation of

H_2O_2 is restricted by the action of CAT and APX which convert H_2O_2 to H_2O (Singh *et al*., 2015).

Non enzymatic antioxidants are ascorbate, which acts as a reductant for peroxidases, tocopherol (located in cell membranes and plays an important role in the scavenging of lipid peroxyl radicals) and carotenoids are known to quench singlet oxygen and minimize its formation by receiving excess energy from the excited chlorophyllcontribution to ROS scavenging under salt stress is unclear(Abogadallah, 2010).

3. Statement of the problem

Salinity effect is one of the major problems in crop production in many areas of the world including Ethiopia. It represents the main obstacle that limits the agricultural production. Cowpea is an important multifunctional crop which may be affected by salinity when cultivated in saline soil environment. It was imperative to determine the ill effects of salinity and understanding of salt tolerance mechanism. At the same time, SA –an important plant hormoneis recognized as an endogenous regulator in many plant growth and physiological processes under various kind of abiotic and biotic stress. SA as a chemical messenger plays an important role in salt tolerance and or mitigation of salinity stress of certain crop and vegetables plant species. Therefore, it is important to determine the role of SAunder salt stress among cowpea cultivars.

4. Hypothesis

H0: salicylic acid cannot induceand altered growth and physiological parameters of three cowpea cultivars grown under salinity stress.

H1: Salicylic acid can induce and altered growth and physiological parameters of three cowpeacultivars grown under salinity stress.

5. Objectives of the study

5.1. General objective

The main objective of the present study was to understand salicylicacid induced alteration in growth and physiological attributesofcowpeacultivars grown under salinity stress.

5.2. Specific objectives

- To determine the cow pea cultivars growth performance as affected by SA under salt stress condition.
- To determine the cow pea cultivars physiological performance as affected by SA under salt stress condition.

6. Significance of the study

Cowpea [*Vigna unguiculata* (L.)Walp.]is one of the multifunctional crop and a traditional source of livelihood to many rural African populations. A wide range of variation exists among different crop plants and their cultivars may be utilized gainfully for identifying and developing the salt-tolerant candidates. Given this, three cowpea cultivars were selected for study of their response to salt stress, assuming that they could show differential capacity of tolerance to salinity. In addition, SA have shown the ameliorative role under salinity. Thus,this investigation able to determine the role of SAagainst salt-stress conditionamong three cowpea cultivars to identify the tolerant cultivar and could be base for further study.

7. Limitation of the study

To conduct detail study on the proposed topic there were some limitations such as time limit, lack of chemical, instrumentation and glass house (controlled environment) facility.

8. Materials and Methods

8.1. Description of study area

Experiments were conducted under open conditions during the winter (December to March) of 2016 –2017 at the Tewodros campus of University of Gondar. The annual average of daily relative humidity is lowest occurring in January and February and highestin July (Husen*et al*., 2016).However;during the entire experimental no rainfall took place.

Location		Avg. Temperature (°C)		Precipitation / annum	Avg. of daily relative humidity	
N	E	Max.	Min.		Max.	Min.
12°35' 14.19"	37° 26' 29.53"	27	16	1161mm	79%)	40%

8.2. Plant material and pot media growth

Seeds ofCowpeawithin three accessions cultivars (ILRI 9334, ILRI 9333 and ILRI 1114) were obtainedfrom Gondar AgriculturalResearch Centre, Gondar, Ethiopia. Healthy cowpea seeds (n =12 for each treatment) were selected for uniformity by choosing those of equal size and samecolor. The selected seeds were washed in distilled water. Seed surface were sterilized with 80% ethanol for 15 min, and again washed thoroughly using distilled water. Thereafter, clean seeds were dipped in distilled water for 12 hours and then sown in plastic tray containing 75% soil and 25% farmyard manure (FYM) for germination. After two weeks of germination, uniform seedlings were chosen and transferred separately to plastic pots (10cm diameter x 18cm height) filled with 2.4 kg soil and 0.80kg FYM in 3:1 ratio ofSandy loam soil, contained 56% sand, 11.44% clay and 32.56% silt; and had pH 6.92; the seedlingswere sown at a depth of 2 cm and irrigated daily with tap water for the next 2 weeks with 100% field capacity (FC), supposedly a period of plant acclimatization;each pot contained one seedling only.

8.3. Experimental design

Afteracclimatization and some growth stage, two-month-old seedlingswere divided in 8 groups to assess the role of SA and salt stresstreatments (Table 1). The pots were arranged in a simplerandomized design,prior to the commencement of treatments.

SA concentration (1mM) was selected; as earlier proved this concentration is beneficial for growth and developmental process under laboratory conditions(Singh *et al*., 2015).SA was initially dissolved in dropsof ethanol (2%) and the final volume was reached using deionized water. Foliar spray of 1Mm SA was donedailyfor 15days. All sprays were applied in the morning (8:00-9:00AM) with a hand sprayer, which occurred after covering the soil surface in order to omission of SA interfering via soil.

Salinity treatments were established from deionized water and sodium chloride (NaCl) as solution of 0, 50, 100, and 150 mM ofNaCl. The salt concentrations used were within the range found in water used for irrigation purposes. Salt treatments were given through irrigation on alternate days for aperiod of 10 days on (2^{nd},4^{th},6^{th}, 8^{th} and 10^{th} day) to each cultivar. Each treatment was replicated four times and each replicate included threepots. Four replications per treatment and 3 plants per replicationof three culivar were arranged in the following (T1-T8) treatments (Singh *et al*., 2015).

Table 1 : Arrangement of treatments for each cultivar

Treatment	Description
T_1	0 mM NaCl, without SA
T_2	0 mM NaCl, with 1mM SA
T_3	50 mM NaCl, without SA
T_4	50 mM NaCl, with 1mM SA
T_5	100 mM NaCl, without SA
T_6	100 mM NaCl, with 1mM SA
T_7	150 mM NaCl, without SA
T_8	150 mM NaCl, with 1mM SA

Seedlings wereallowed to adjustwith soil salinity for about a week; and thereafter watered regularly with100% field capacity (FC). After about two weeks of plant growth in the presence of NaCl, sampling was done. At that time, all cultivars were threemonths and two-week-old.

8.4. Plant growth

8.4.1. Measurement of length: The root and shoot length of each cultivars were measured in cm.

8.4.2. **Number of branches and leaves**: The number of leaves and branches of each cultivar were counted.

8.4.3. **Stem basal diameter**:Ground-line basal diameter (mm) of stem of each cultivar was measured with electronic digital caliper.

8.4.4. **Leaf area**: The area (mm^2) withlength (mm)and width (mm)of individual leaves of each cultivarwas measured using AM300 leaf area meter (ADC Bio Scientific Limited, U.K.).

8.4.5.Plant biomass production

Roots, stems and leaves of each cultivars and treatments were separated to obtain their total dry mass (g) on a CY510 electronic digital balance (Citizen Scale, Poland). All samples were dried in an ovum at 80^0c for a period of 48 hours.

8.6. Measurement of plant water status

Water status of leaf was determined for each treatment (T1-T8) and cultivarsby measuring the relative water content (RWC) of fully-developed leaves. Leaves were weighed (FW) and then kept in distilled water overnight in the dark, to obtain their turgid weight (TW). It wasthen oven-dried at 80°C for 24 and weighed again to obtain dry weight (DW). RWC were determined as (Husen *et al*., 2016)

$$RWC\ (\%) = (FW\text{-}DW) / (TW\text{-}DW)] \times 100.$$

8.6. Measurement of chlorophyll fluorescence (Fv/Fm)

Chlorophyll fluorescence of leaves was recorded in the forenoon **(**10 to 11 AM**)** for each treatment with the help of portable Multi-Mode OS5P Chlorophyll Fluorometer (Opti-Sciences, Inc., USA). Prior to fluorescence measurements, the upper surface of leaf was pre-darkened with leaf clips for 30 minutes to ensure complete relaxation of all reaction centres. The basal non-variable chlorophyll fluorescence (Fo), maximal fluorescence induction (Fm), and variable fluorescence (Fv) were determined. The maximum quantum yield of PSII efficiency (Fv/Fm) was estimated by the ratio Fv/Fm = (Fm –Fo)/ Fm (Genty*et al*., 1989).

8.7. Data analysis

Analysis of variance was performed for all growth and physiological traits usingSAS version 9.1software. Means were separated by using Duncan's test. Data were subjected to two-way analysis of variance (ANOVA) to determine significant difference among the treatments, cultivars and their ineraction. Means were compared at significance level of $P < 0.05$.

9. Result

9.1.Plant growth

The effect of SA foliar application on growth characteristics of cowpea cultivars namely, ILRI1114, ILRI9333 and ILRI9334 grown under control (without SA and salinity) and under salt-stress conditions (with and without SA) was assessed; and presented in (Table 2).

In the cultivar ILRI1114, a significantdecline in number of leaves was observed with increasing salinityconcentration in a dose dependent manner (Table2 and 5). In terms of percent variation, in comparison to control, the decreasewas 24%, 28% and 46% under 50mM, 100mM and 150mM NaCl, respectively. However, in comparison to control, the foliar application of 1mM SA reduced the negative impact of salinityby8%, 8% and 39%under50mM, 100Mm and 150mMNaCl, respectively. In the ILRI9333, in comparison to control, the numbers of leaves were declined by 27%, 40% and45%, at 50mM, 100Mm and 150mM NaCl, respectively. SA application has shown less reduction, thus, in comparison to control, the percent variation was 11%, 28% and 40% at 50mM, 100Mm and 150mM NaCl, respectively. In the ILRI9334, the number of leaves was declined by 17%, 25% and 32%under 50mM, 100Mm and 150mM NaCl, respectively. However, SA application has shown less reduction of number of leaves. Thus, in comparison to control, this improvement was 15%, 33% and 32% at 50mM, 100Mm and 150mM NaCl over control, respectively. Overall, in terms of number of leaf cultivar ILRI9334 was less affected, in comparison to ILRI1114 and ILRI9333.

In the cultivar ILRI1114, a significant decline in shoot length was observed under the highest concentration of salinity (Table 2). In terms of percent variation, in comparison to control, the decrease was 26%. However, in comparison to control, the foliar application SA reduced this negative impact of salinity by 16%. In the ILRI9333, in comparison to control, the shoot length was declined by 29% at 150mM NaCl. SA application has shown less reduction, thus, in comparison to control, the percent variation was 18% at 150mM NaCl. Further,all three studied cultivars have shown insignificant varaiotion at 50mM and 100mM NaCl. However, cultivar ILRI9334 has also exhibited insignificant varaiton at higher level of salinity; here in all level of salinity stress, SA application was found to be increased the shoot length.Overall, in terms of shoot length cultivar ILRI9334 was less affected, in comparison to ILRI1114 and ILRI9333(Table 2).

In the cultivar ILRI1114, under higher dose salinity concentration there was a significant decline in stem basal diameter (Table 2). In terms of percent variation, in comparison to control, the decrease was 44% and 51% at 100mM and150mM NaCl, respectively. SA application has reduced the lossby 28% and 35% at 100mM and 150mMNaCl, respectively. In the ILRI9333, in comparison to control, stem basal diameter was declined by 35% and 36% under100mMand 150mM NaCl, respectively. SA application has improved basal diameter increasement, and thus, the loss was 32% and 30% at 100mM and 150mM NaCl, respectively. Cultivar ILRI9334, the stem basal diameter was declined by 30% and 37%under 100mM and 150mM NaCl, respectively. However, SA application has shown less reduction of basal diameter. Thus, in comparison to control, this decrease was 24% and 22% under 100mM and 150mM NaCl, respectively. Overall, in terms of stem basal diameter cultivarcultivar ILRI9334 was less affected, in comparison toILRI1114 and ILRI9333.

All three cultivars, grown under control (without SA and salinity) and under salt-stress conditions (with and without SA) have shown insignificant variation for the number of branch and root length in comparison to control plants (Table 2).

Leaf area was reduced significalty in cultivar ILRI1114in a dose dependent manner (Table 2). In terms of percent variation, in comparison to control, the decrease was 15% and 21% at 100mM and 150Mm NaCl, respectively. However, at 50Mm NaCl, the variation was insignificant. Further, in comparison to control, the foliar application SA reduced the negative impact of salinity by 4% and 21% under 150mM and 100Mm NaCl, respectively.However, cultivar ILRI9333, in comparison to control, both salt stress and SA application had showninsignificant variation of the leaf area. In the ILRI9334, the leaf area was declined by 21% and 42% under 100mM and 150Mm NaCl, respectively. However, SA application has shown stimulatroty effect and thus reduction of leaf area was less under different level of salt-stress condition. Therefore, in comparison to control, this decrease was 20% and 24% at 100mM and 150mM NaCl, respectively. Overall, in terms of leaf area production, the cultivar ILRI9334 was less affected, in comparison to ILRI1114 and ILRI9333.

At higher level of salinity, cultivar ILRI1114 has also shown reduction in leaf width (Table 2). Thus, leaf width was reduced by 18% at 150mM NaCl, in comparison to control. SA application has improved this situation and leaf width was reduced by 10%. However, in comparison to control, ILRI9333 have shown slightreduction at various level of salinity stress

by 2%, 4% and 2%.SA application has strongly improved this reduction, thus in comparison to control, leaf width was increased by19%, 21% and 12% at 50mM, 100Mm and 150mM NaCl, respectively. Cultivar ILRI9334, the leaf width was significantly declined at higher level of salinity by 15%, in comparison to control planls. However, SA application was found to be promotive. Thus, in comparison to control, leaf width was totalty recovered and showed 18%variation at 150mM. Overall, in terms of leaf width cultivar ILRI9333 was less affected, in comparison to ILRI1114 and ILRI9334.

In the cultivar ILRI1114, a significant decline in leaf length was observed by2%, 8% and 16% at 50mM, 100Mm and 150mM NaCl, respectively (Table 2).However, in comparison to control, the foliar application of 1mM SA reduced the negative impact of salinity by 4%, 13% and 13% at 50mM, 100Mm and 150mM NaCl, respectively. Cultivar ILRI9333 have shown more reduction at higher salinity-stress condtion (15%),in comparison to control, while this impact was recovered by SA application. Further, cultivar ILRI9334, leaf length was declined by 12%, 14% and15%, at 50mM, 100Mm and 150mM NaCl, respectively over control.SA application has restored leaf length by 7%, 6% and 6%. Overall, in terms of leaf length cultivar ILRI9333 was less affected, in comparison to ILRI1114 and ILRI9334.

Table 2: Effects of salicylic acid treatments on the growth attributes of three cultivars cow peagrown under salt-stress conditions

Cultivars	Treatments	Numberof Leaves	Number of Branch	Shoot length (cm)	Stem basal diameter (mm)	Root length (cm)	Leaf area (mm^2)	Leaf width (mm)	Leaf length (mm)
ILRI1114	0 mM NaCl, without SA	43.83±2.80^{ba}	1.67±0.29^{dc}	46.25±2.76^{bc}	6.25±0.46^{b}	9.67±0.68^{a}	2919.5±176.83^{c}	49.4±1.96^{bc}	59.25±2.13^{cd}
	0 mM NaCl, with 1mM SA	50.00±2.80^{a} (14.07)	2.42±0.29^{bac} (45.51)	54.75±2.76^{a} (18.38)	8.42±0.46^{a} (34.72)	9.500±0.68^{a} (1.76)	4155.42±176.83^{a} (42.33)	54.42±1.96^{a} (10.16)	77.49±2.13^{a} (30.78)
	50 mM NaCl,without SA	33.16±2.80^{dc} (24.32)	2.33±0.29^{bc} (39.52)	51.17±2.76^{bac} (10.64)	5.08±0.46^{cb} (18.72)	8.73±0.68^{a} (9.72)	2919.42±176.83^{c} (0.01)	44.671.96^{bdc} (9.57)	58.25±2.13^{ed} (1.69)
	50 mM NaCl, with 1mM SA	40.33±2.80^{bc} (7.98)	2.83±0.29^{bacd} (69.46)	48.00±2.76^{ba} (3.78)	5.17±0.46^{cb} (17.28)	8.75±0.68^{a} (9.51)	3043.83±176.83^{cb} (4.2)	48.50±1.96^{bdc} (1.86)	61.75±2.13^{cb} (4.22)
	100 mM NaCl,without SA	31.41±2.80de (28.31)	1.66±0.29^{bcd} (0.01)	42.91±2.76^{c} (7.2)	3.50±0.46^{d} (44)	9.47±0.68^{a} (2.07)	2493.83±176.83^{d} (14.58)	46.00±1.96^{dc} (7.39)	54.17±2.13^{e} (8.59)
	100 mM NaCl,with 1mM SA	40.33±2.80^{de} (7.98)	2.83±0.29^{bacd} (69.46)	50.58±2.76^{ba} (9.36)	4.50±0.46^{dc} (28)	10.50±0.68^{a} (8.59)	3523.33±176.83^{b} (20.69)	52.17±1.96^{ba} (5.31)	67.53±2.13^{b} (12.79)
	150 mM NaCl,without SA	23.75±2.80^{e} (45.81)	1.25±0.29^{cd} (25.15)	34.08±2.76^{d} (26.31)	3.05±0.46^{cd} (51.2)	8.33±0.68^{a} (13.86)	2306.75±176.83^{d} (20.99)	40.54±1.96^{d} (17.88)	50.00±2.13^{cb} (15.61)
	150 mM NaCl,with 1mM SA	26.58± 2.80^{de} (39.36)	1.58±0.29^{d} (5.39)	38.75±76^{d} (16.22)	4.08±0.46^{cd} (34.72)	8.67±0.68^{a} (10.34)	3438.83±176.8^{b} (4.08)	54.42±1.96^{a} (10.16)	66.83±2.12^{b} (12.79)
ILRI9333	0 mM NaCl, without SA	46.50±2.80^{a}	1.67±.29^{bc}	32.50 ±4.28^{bc}	6.25±0.71^{b}	7.5±1.05^{c}	2563.58±176.83^{b}	41.63±3.04^{d}	49.90±3.29^{bc}
	0 mM NaCl, with 1mM SA	50.33±2.80^{a} (8.24)	2.1±29^{ba} (25.74)	40.08±2.76^{a} (23.32)	8.42±.46^{a} (34.72)	10.50±0.68^{a} (30.0)	3223.33±176.83^{ba} (25.74)	46.75±1.96^{bdac} (12.30)	68.51±2.13^{a} (37.32)
	50 mM NaCl, without SA	34.16±2.80^{cb} (26.53)	2.08±0.29^{ba} (24.55)	35.5±2.76^{ba} (9.23)	5.12±0.46^{cb} (18.08)	9.83±0.68^{ba} (31.1)	2563.58±176.83^{b} (0.00)	42.58±1.96^{dc} (2.28)	59.64±2.13^{b} (19.54)
	50 mMNaCl, with1mM SA	41.33±2.80^{cba} (11.12)	2.16±0.29^{ba} (29.94)	33.46±2.88^{bc} (2.31)	5.08±.48^{cb} (18.72)	11.25±0.72^{a} (51.41)	2748.64±184.63^{b} (7.22)	49.5±2.05^{ba} (18.91)	57.1±2.22^{b} (14.43)
	100 mM NaCl, without SA	27.83±2.80^{cd} (40.15)	1.58±0.29^{bc} (5.39)	28.50±2.76^{cd} (12.31)	3.83±0.46^{c} (35.43)	8.42±0.68^{bc} (12.27)	2405.17±176.83^{b} (6.18)	43.68±1.96^{bdc} (4.80)	55.01±2.13^{bc} (10.24)

	100 mM NaCl, with 1mM SA	33.58±2.80^{cb} (27.78)	1.58±0.29bc (5.39)	29.33±4.28^{bcd} (9.75)	4.25±0.72^{c} (32)	10.92^{a}±1.05 (45.6)	3065.80±273.94^{ba} (19.58)	50.38±3.04^{a} (21.02)	57.35±3.23^{b} (14.93)
	150 mM NaCl, without SA	25.58±2.80d (44.99)	1.00±0.29^{c} (40.13)	21.33±3.91^{d} (28.98)	4.0±0.66^{c} (36)	10.0±0.96^{ba} (33.33)	2621.1±193.71^{b} (2.24)	42.56±2.15^{dc} (2.2)	66.95±2.33^{a} (34.17)
	150 mM NaCl, with 1mM SA	27.58±2.82cd (40.15)	1.08±0.291^{c} (35.33)	26.6±3.03^{d} (18.15)	4.08±0.51^{c} (30.04)	9.75±0.74^{ba} (30)	3047.61±250.07^{ba} (18.88)	48.57±2.77^{bac} (11.87)	50.54±3.00^{c} (1.3)
ILRI9334	0 mM NaCl, without SA	30.58±2.80^{c}	1.91±0.29^{cb}	41.83±2.76^{dc}	7.17±0.46^{a}	8.08±0.68^{a}	2504.00±176.83^{bc}	41.56±1.96^{edc}	59.86±2.13^{a}
	0 mM NaCl, with 1mM SA	34.50±2.80bc (12.82)	3.08±0.29^{a} (61.25)	54.75±2.76^{a} (30.89)	6.41±0.46^{ba} (10.46)	8.48±0.68^{a} (4.95)	2885.33±176.83^{ba} (15.23)	45.85±1.96^{bac} (10.32)	62.55±2.13^{a} (4.5)
	50 mM NaCl, without SA	25.50±2.80^{ba} (16.66)	1.91±0.29^{cb} (0.00)	41.83±2.76^{bdc} (26.89)	6.00±0.46bac (16.32)	7.25±0.68^{ba} (10.27)	2276.42±176.83^{dc} (9.09)	43.46±1.96^{bdc} (4.42)	52.86±2.13^{b} (11.66)
	50 mM NaCl,With 1mM SA	35.33±2.80^{cbd} (15.13)	2.08±0.29^{b} (7.84)	52.58±2.76^{ba} (25.70)	6.41±0.46^{dc} (10.46)	6.10±0.68^{b} (24.50)	2448.25±176.83^{c} (15.16)	37.92±1.96^{ef} (8.76)	64.24±2.12^{a} (7.32)
	100 mM NaCl,without SA	23.08±2.80^{ba} (24.53)	1.42±0.29^{db} (25.93)	40.25±2.7^{dc} (4.37)	5.00±0.46^{dc} (30.26)	6.15±0.68^{b} (23.89)	1977.23±176.83^{de} (21.14)	38.58±1.96^{edf} (7.29)	51.25±2.13^{b} (14.38)
	100 mM NaCl,with mM SA	40.75±2.80^{d} (33.25)	1.91±0.29^{cb} (0.00)	48.42±2.76^{bdac} (15.75)	5.41±0.46^{dc} (24.41)	6.15±0.68^{b} (23.76)	3008.00±176.83^{a} (20.13)	47.42±1.96^{ba} (14.10)	63.42±2.13^{a} (5.95)
	150 mM NaCl,without SA	20.66±2.80^{a} (32.43)	1.08±0.29^{c} (43.46)	38.92±2.76^{de} (6.96)	4.5±0.46^{d} (37.23)	7.77±0.68^{a} (3.84)	1746.82±176.83^{e} (42.25)	34.42±1.96^{f} (17.18)	50.75±2.13^{b} (15.22)
	150 mM NaCl,with 1mM SA	40.25±2.80^{d} (31.62)	1.42±0.29^{db} (25.65)	46.25±2.76^{bdc} (10.57)	5.61±0.46bc (21.76)	6.28±0.68^{b} (22.278)	3108.83±176.83^{a} (24.16)	49.08±1.96^{a} (18.09)	63.17±2.13^{a} (5.53)

Each value represents the mean ± SE of four replicates. Values followed by the same letter indicate no significant differences at P <0.05 level according to the Duncan test. Values withinparenthesis are percent variation as obtained from the control plants of respective cultivars.

Plant biomass production

All three cultivars, grown under control (without SA and salinity) and under salt-stress conditions (with and without SA) have shown alomost insginifcant varariation at lower concentration (50mM and 100Mm NaCl) while at higher concentration (150mM NaCl) of salinity stress these variations was more. Furthermore, in almost level of salinity stress, SA application was found to be increased the plant bimoss production in all three cultivars.Overall, in terms ofbiomass production cultivar ILRI9334 was less affected, in comparison toILRI1114 and ILRI9333 (Table 3).

Table 3: Effects of salicylic acid treatments on the biomass production of three cultivars cow peagrown under salt-stressconditons

Cultivars	Treatments	Stem (g)	Leaves (g)	Root (g)	Whole plant (g)
ILRI1114	0 mM NaCl, without SA	5.73±0.52^{bc}	3.28±0.392^{b}	0.78±0.08^{a}	9.7±0.56^{bac}
	0 mM NaCl, with 1mM SA	6.69±.57^{bca} (16.73)	3.57±0.253b (8.8)	0.85±0.08^{a} (8.97)	11±0.56^{a} (13.4)
	50 mM NaCl, without SA	5.13±0.52^{bc} (16.18)	2.90±0.253^{cb} (9.06)	0.83±0.08^{a} (6.41)	8.47±0.56^{bc} (12.88)
	50 mM NaCl, with 1mM SA	6.12±0.73^{ba} (12.56)	2.86±0.25^{cb} (12.8)	0.85±0.08^{a} (8.97)	9.16±0.73^{bc} (5.56)
	100 mM NaCl, without SA	5.52±.52^{bc} (3.66)	2.45±0.264^{c} (25.3)	0.81±.08^{a} (3.85)	8.8±1.36^{c} (9.27)
	100 mM NaCl, with 1mM SA	6.49±0.52^{a} (13.26)	3.0±0.392^{cb} (8.54)	0.82±.08^{a} (5.12)	9.47±.77^{bc} (2.37)
	150 mM NaCl, without SA	3.95±.52^{d} (31.06)	2.4±.277^{c} (26.82)	0.55±.109^{c} (29.48)	6.9±.79^{d} (28.87)
	150 mM NaCl, with 1mM SA	6.0±0.52^{ba} (4.71)	3.00±0.358^{cb} (8.54)	0.58±.084^{ca} (25.64)	9.58±0.61^{bc} (1.24)
ILRI9333	0 mM NaCl, without SA	7.87±0.518^{bac}	2.86±0.25^{a}	0.6±0.12^{c}	11.33±0.56^{ba}
	0 mM NaCl, with 1mM SA	7.07±0.518^{bdac} (10.17)	2.93±0.25^{a} (4.64)	0.57±0.08^{ac} (5)	10.57±0.56^{ba} (6.7)
	50 mM NaCl, without SA	6.67±0.518^{bdc} (15.25)	2.53±0.25^{ba} (11.54)	0.59±0.08^{c} (1.67)	9.79±0.56^{ba} (13.59)
	50 mM NaCl, with 1mM SA	8.43±0.518^{a} (7.11)	3.03±0.25^{a} (8.21)	0.6±0.08^{c} (0)	9.32±0.56^{b} (17.74)
	100 mM NaCl, without SA	6.16±0.518^{dc} (21.73)	2.36±0.25^{ba} (17.48)	0.56±0.08^{ac} (5)	9.23±0.56^{b} (18.53)
	100 mM NaCl, with 1mM SA	8.20±0.518^{ba} (15.98)	3.06±0.25^{a} (9.29)	0.57±0.119^{ac} (5)	10.2±0.56^{a} (4.0)
	150 mM NaCl, without SA	6.08±0.518^{d} (15.02)	2.22±0.25^{b} (22.37)	0.55±.109^{ac} (8.3)	10.1±0.56^{a} (9.97)

	150 mM NaCl, with 1mM SA	8.07±0.518^{ba} (14.14)	2.73±0.25^{ba} (4.54)	0.58±.084^{c} (3.3)	9.47±0.56^{a} (16.42)
ILRI9334	0 mM NaCl, without SA	7.0±0.80^{bc}	2.2±0.253^{bc}	0.32±0.08^{b}	10.67±0.862^{b}
	0 mM NaCl, with 1mM SA	7.2±0.52^{bc} (2.85)	2.96±0.25^{ba} (34.54)	0.59±0.08^{a} (84.38)	13.26±0.56^{a} (24.27)
	50 mM NaCl, without SA	7.2±0.518^{bc} (2.85)	2.2±.277^{bc} (0.0)	0.52±0.08^{ba} (62.5)	9.90±0.56^{cb} (7.23)
	50 mM NaCl, with 1mM SA	7.25±0.541^{bcd} (3.6)	2.19±0.25^{bc} (0.46)	0.56±0.08^{ba} (75)	10.±0.58^{b} (6.28)
	100 mM NaCl, without SA	6.93±.518^{bac} (10.)	2.16±0.25^{bc} (1.8)	0.55±0.08^{ba} (71.88)	9.64±0.56^{bc} (9.65)
	100 mM NaCl, with 1mM SA	6.97±0.80^{bc} (4.3)	2.19±.25^{bc} (0.45)	0.62±0.08^{a} (75)	9.78±.79^{c} (8.34
	150 mM NaCl, without SA	6.12±0.73^{ba} (12.56)	2.00±0.358^{bd} (9.1)	0.58±0.08^{a} (81.25)	9.78±.79^{c} (8.34)
	150 mM NaCl, with 1mM SA	6.69±.57^{bca} (4.43)	2.12±0.25^{bd} (8.)	0.58±0.08^{a} (81.25)	9.39±0.61^{c} (12)

Each value represents the mean ± SE of four replicates. Numbers followed by different letters indicate significant differences ($P < 0.05$) according to the Duncan test. Values within parenthesis are percent variation as obtained from the control plants of respective cultivars.

9.2.Leaf relative water content (RWC) and chlorophyll fluorescence (Fv/Fm)

The effect of SA foliar application on RWC and Fv/Fmof cowpea cultivars namely, ILRI1114, ILRI9333 and ILRI9334 grown under control (without SA and salinity) and under salt-stress conditions (with and without SA) was assessed; and presented in (Table 4).

Plants grown under highest salt concentration showed significant decrease on RWC. Thus, at 150 mM NaCl, the reduction of RWC was 30% and 29% for cultivar ILRI1114 andILRI 9333, repectively, in comparison to control plants. Like other parameters RWC was also markedly improved due to SA application (Table 4). Overall, in terms ofon RWC cultivar ILRI 93334 was less affected, in comparison to ILRI1114 and ILRI9333.

Value of Fv/Fm have shown slight variation but these were insignificant for ILRI1114, ILRI9333 and ILRI9334 cultivars grown under control (without SA and salinity) and under salt-stress conditions (with and without SA) (Table 4).

Table 4: Effects of salicylic acid treatments on the leaf relativewater content and chlorophyll fluorescence of three cultivars cow peagrown under salt-stress conditons

Cultivars	**Treatments**	**Leaf relative water Content (%)**	**Chlorophyll fluorescence (*Fv/Fm*)**

ILRI1114	0 mM NaCl, without SA	55.49±3.215^{cb}	0.71±12.98^{a}
	0 mM NaCl, with 1mM SA	74.41±3.22^{a} (34.09)	0.71±12.99^{a} (0.00)
	50 mM NaCl without SA	56.88±3.2^{cb} (2.48)	0.65±12.99^{ba} (8.45)
	50 mM NaCl with 1mM SA	60.170±3.215^{b} (8.41)	0.71±12.99^{a} (0.00)
	100 mM NaCl without SA	51.015±3.22^{cd} (8.07)	0.64±12.99^{ba} (9.85)
	100 mM NaCl with 1mM SA	54.37±3.215^{cd} (2.02)	0.69±12.99^{a} (2.82)
	150 mM NaCl without SA	38.96±3.215^{e} (29.80)	0.59±12.99^{b} (16.9)
	150 mM NaCl with 1mM SA	46.58±3.22^{ed} (16.06)	0.69±12.99^{a} (2.82)
ILRI9333	0 mM NaCl, without SA	45.19±4.98^{cd}	0.65±20.12^{ac}
	0 mM NaCl, with 1mM SA	65.73±3.22^{a} (45.45)	1.12±12.99^{b} (72.31)
	50 mM NaCl without SA	48.30±3.22^{cbd} (6.64)	0.67±12.99^{a} (3.08)
	50 mM NaCl with 1mM SA	53.96±3.36^{cb} (19.45)	0.77±18.37^{a} (18.46)
	100 mM NaCl without SA	43.65±3.22^{d} (7.12)	0.62±12.99^{ac} (4.62)
	100 mM NaCl with 1mM SA	55.80±4.98^{b} (23.48)	0.65±20.12^{ac} (0.00)
	150 mM NaCl without SA	32±4.55^{e} (29.19)	0.62±13.66^{ac} (4.62)
	150 mM NaCl with 1 mM SA	54.53±3.52^{b} (20.67)	0.69±14.23^{a} (6.15)
ILRI9334	0 mM NaCl, without SA	44.32±3.22^{bc}	0.75±12.99^{ba}
	0 mM NaCl, with 1mM SA	58.85±3.22^{a} (32.78)	0.74±12.99^{ba} (1.33)
	50 mM NaCl without SA	42.13±3.22^{bc} (0.43)	0.74±12.99^{bac} (1.33)
	50 mM NaCl with 1 mM SA	49.93±3.22^{ba} (12.66)	0.74±12.99^{bc} (1.33)
	100 mM NaCl without SA	38.79±3.22^{c} (12.47)	0.70±12.99^{c} (8)
	100 mM NaCl with 1mM SA	46.44±3.22^{bc} (4.78)	0.74±12.99^{ba} (1.33)
	150 mM NaCl without SA	41.7±3.22^{bc} (5.91)	0.74±12.99^{bac} (1.33)
	150 mM NaCl with 1mM SA	45.77±3.22^{bc} (3.27)	0.78±12.99^{a} (4)

Each value represents the mean ± SE of four replicates. Numbers followed by different letters indicate significant differences ($P < 0.05$) according to theDuncantest. Values within parenthesis are percent variation as obtained from the control plants of respective cultivars.

Table 5: Analysis of variance results on the effect of cultivars, salt treatments and their interaction for growth, relative water content and chlorophyll fluorescence of cowpea

Parameters	**Cultivars**			**Treatments**			**Cultivars x Treatments**		
	MS	P- Value <0. 05	P-value significances	MS	P-value<0.05	P-value significances	MS	P-value<0.05	P-value significances
Number of leaves	1334.23	<.0001	*	1334.22	<.0001	*	509.51	<.0001	*
Number of branch	4.16	0.0180	*	16.48	0.0180	*	16.48	0.1251	-
Leaf area (mm)	8525609.13	<0.0001	*	1167970.62	<.0001	*	5571069.62	0.0002	*
Leaf length (mm)	618.67	<0.0001	*	1013.41	<.0001	*	412.88	<.0001	*
Leaf width (mm)	808.57	<0.0001	*	451.7	<.0001	*	89.84	0.0265	*
Stem basal diameter	7.61	0.0668	-	56.94	<.0001	*		-	-
Shoot length (cm)	7581.15	<0.0001	*	1175.86	<.0001	*	125.05	0.1715	-
Root length (cm)	200.52	<0.0001	*	8.4251079	0.1729	-	14.11	0.0025	*
Relative water content (%)	1582.58	<0.0001	*	2323.18	<.0001	*	341.82	0.0008	*
Leaf dry mass	8.55	<0.0001	*	1.79	0.0258	*	3.03	<.0001	*
Stem dry mass	130.48	<0.0001	*	3.2590498	0.4229		12.48	<.0001	*
Root dry mass	2.41	<0.0001	*	0.21	0.0061	*	0.05	0.8025	-
Chlorophyll fluorescence	0.68	<0.0001	*	0.32	<0.001	*	0.007	0.39	_

MSS: mean square value,*significant at p<.05 at; p<0.05and- is insignificant

10. Discussions

Salinity is a global problem that limits the growth and plant productivity of all kind of vegetation and it is going to increase day by day. Further, the beneficial role of various plant hormones in signaling network, developmental and adaptation processes of various plant species against biotic and abiotic stresses has been recognized since long. In recent years, role of salicylic acid (SA) for improved plant growth and production has received much attention. Thus, the present study examined the ameliorative role of salicylic acid (SA) in salinity-induced stress in *Vigna unguiculata* cultivars, namely ILRI1114, ILRI9333 and ILRI9334. The results showed that growth and biomass attributes were gradually decreased under salt-stress conditions. In all cultivars, significant reduction growth and biomass attributes was recorded under higher level salinity but cultivar ILRI9334performed better under salinity stress. The present results are in the same line of earlier finding in mustard (Nazar *et al*., 2015),pea(Husen *et al*., 2016)faba bean(Husen *et al*., 2017) and tomato(Albaladejo*et al*., 2017)under stress. It has been also reported that saline environment in the soil influences water imbibition by roots due to low osmotic potential of the substrate, besides hampering the phenomena of photosynthesis, accumulation of compatible solutes, nutrient homeostasis, protein synthesis and modulation of enzymatic and non-enzymatic antioxidants (Qureshi *etal*., 2013; Bagheri *et al*., 2015; Yousuf *et al*., 2015a, 2015b). Therefore, cowpea cultivars growth and biomass production was decline; and this might be due to reduced leaf area, imbalance in plant water status and reduced production of photoassimilates. Moreover, application of 1mM SA significantly improved growth and biomass parameters of cowpeaplants under various salt-stress conditions. These results are consistent to previous investigation, for instance SA ameliorated the negative effects of growth and related parameters in maize (Khodary, 2004), barley (El-Tayeb, 2005), mungbean (Khan *et al*., 2014) and mustard (Nazar *et al*., 2015). Some authors (Wang and Li, 2006; Aftab *et al*., 2011) have also linked the role of SA in membrane protection and thus plant tolerance capacity might be increased under salt-stress condition.

Leaf relative water content (RWC) has been extensively used in the classical literature to determine the physiological water status of plants. Herein results exhibited that as the salt concentration was increased, the RWC decreased in all cultivars; this indicates a loss of turgor that results in limited water availability for cell-extension processes. Previous studies have also shown decreased leaf RWC due to salt stress (Sekmen *et al*., 2007). However,

cultivar variations were observed and found that ILRI9334 performed better under salinity stress. Further, leaf RWC was increased in all cultivars in response to 1mM SA application under salt-stress condition, possibly considered as an adaptive symptom in improving its moistness and sustaining the water balance in response to salinity-induced osmotic stress (Li *et al*., 2014; Rady and Mohamed, 2015). Moreover, the positive improvement of SA induced antioxidant activity and reduction in oxidative stress was further showed by an increase in RWC and growth of cowpea cultivars.

The reduction in the photochemical efficiency of PSII (Fv/Fm) value under stressful environment, duly linked with a decrease in photosynthetic attributes including leaf pigments and biomass production, is used as an indicator for determining the seedling-stock quality (Husen 2013; Getnet *et al*., 2015).Due to salt-stress treatment, Fv/Fm was found to be decreased insignificalty in a dose-dependent manner in all cultivars which indicates that saline condition have some influences the photochemistry of photosynthesis. However, these variations were insignificant. This is parallel to some earlier findings (Kalaji *et al*., 2011; Husen *et al*., 2016, 2017). However, Fv/Fm was increased in all cultivars in response to SA application under salt-stress condition. Reduced of Fv/Fm ratio and non-photochemical quenching coefficient (qN) under salt stress was also observed in tomato plants, whereas both parameters were restored by SA treatments. Nevertheless, Asensi-Fabad and Munné-Bosch (2011) have reported that under the salt-stress condition, the SA-deficient and SA-hyperaccumulating *Arabidopsis*mutants exhibits insignificant difference in chlorophyll contents as well as Fv/Fm ratio (Asensi-Fabad and Munné-Bosch, 2011).

Overall, in all studied cultivars, the growth (number of leaves and branches, shoot and root length, stem basal diameter, leaf area, width and their length), biomass production (leaf, stem, root and total biomass) and some physiological (RWC and Fv/Fm) parameters were markedly declined under salt-stress condition in a dose-dependent manner. However, cultivar ILRI9334 has shown a reasonable tolerance ability followed by ILRI1114 and ILRI9333. Moreover, application of 1mM SA has stimulated the salt-tolerance capacity in all cultivars.

11. Conclusions

The reported investigation was undertaken to study the plant growth, biomass, relative water content and chlorophyll fluorescenceparametersin three cowpea cultivarsdiffering in salt tolerance and to observe the effectiveness of application of 1mM SA in the mitigation of salt stress. In general, cultivar ILRI9334 had shown better tolerance than ILRI1114, ILRI9333 under various levels of salt-stress conditions which was associated with the improved growth, biomass and physiological attributes. Moreover, the application of 1mM SA in alleviating salt-induced stress was more noticeable the cultivar ILRI9334 than ILRI1114 and ILRI9333.

12. Recommendation

At global level, the salt stress has been recognized as a major threat to the agricultural system.External supply of plant growth regulators (PGRs) may trounce the internal PGR deficiency and help the plant resist against stresses. Like many other PGRs such as abscisic acid,auxins, cytokinins, gibberellic acid, brassinosteroid, jasmonates and ethylene; the salicylic acid - a phenolic growth regulator -also offers protection against salinityand many other stresses. Present study has demonstrated that salt stress influenced few growth and physiological parameters among all cowpea cultivars, while application of SA hasalleviated the impact of salinity. Tolerancemechanisms are varied among all cowpea cultivars. To understand this tolerance mechanism more investigations are required at physiological, biochemical and molecular level.

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Appendix

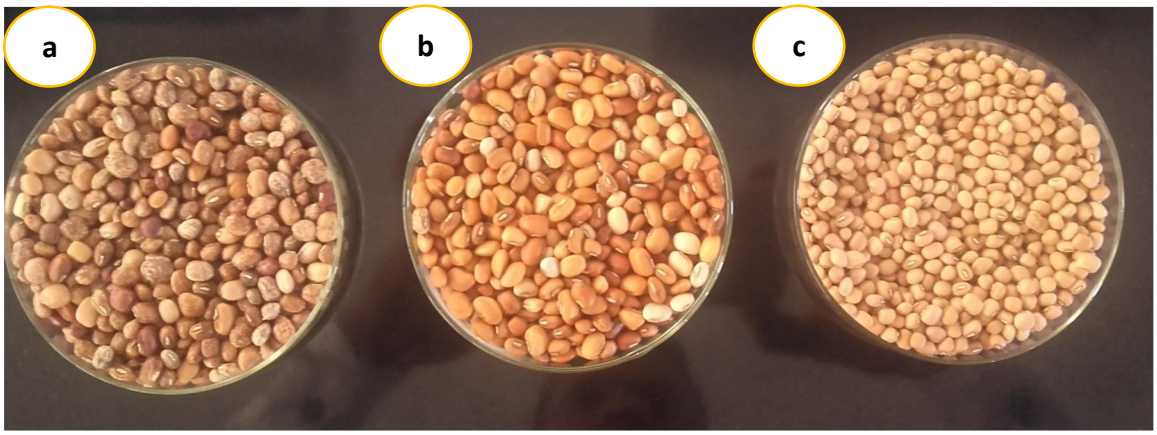

Plate 1 Seeds of cow pea cultivar (a) ILRI 9333, (b) ILRI 1114 and (c)ILRI 9334

Plate2 Other miscellaneous activites

Declaration o f the student and approval of the adviser

I, Ayichesh Hayimrodeclare that this work entitled **"Salicylic Acid Induced Alteration in Growth and Physiological Attributes of Cowpea [*Vigna unguicula* Walp.] Cultivars Grown under Salinity Stress"**is outcome of my own effort and study and that all sources of materials used forthe study have been duly acknowledged. I have produced it independently except for theguidance and suggestion of the research my advisorProfessor Azamal Husen and co-adviser Dr.Getnet Masresha. This study has not been submitted for any degree in this University or any other University. It is offered for the partial fulfillment of the degree ofthe Master of Science in Botanical Sciences.

1 Name of Student Ayichesh Hayimro Signature Date

_____

ID No.GUR/6333/08

2. Name of advisor(s)_____ _____ _____

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